

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:) Group Art Unit: 1646
COX et al.)
Serial No.: 10/031,154)
Filed: January 14, 2002)
Atty. File No.: 4152-3-PUS)
For: "IMMUNOGLOBULIN FUSION)
PROTEINS")

DECLARATION OF
GEORGE COX AND DANIEL DOHERTY
UNDER 37 CFR 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, George Cox and Daniel Doherty, each declare as follows:

1. I am a co-inventor of the above-referenced patent application and am familiar with the application.
2. This Declaration under 37 CFR §1.131 is being submitted in conjunction with a Response to an Office Action mailed on September 25, 2008, the Response being filed herewith.
3. This Declaration provides factual evidence of the conception of the invention as claimed in *at least* Claims 90-94, 96, 102, 104, 105, 130-135 and 139, at a date prior to the July 24, 1998 effective filing date of the material cited in Blumberg, et al., U.S. Patent No. 6,485,726 in support of the rejections under 35 U.S.C. § 103(a), followed by diligence in reduction to practice from a date prior to the effective filing date of Blumberg, et al. (July 24, 1998) to the date of constructive or actual reduction to practice of Claims 90-94, 96, 102, 104, 105, 130-135 and 139. All acts relied upon to establish the dates of conception, diligence and reduction to practice were carried out in the United States.

Evidence of Conception prior to July 24, 2008

As evidence of conception of the invention as claimed in Claims 90-94, 96, 102, 104, 105, 130-135 and 139, at a date prior to July 24, 1998, enclosed as Exhibit A are relevant portions of a grant application that was prepared by us and submitted at a date prior to July 24, 1998.

This grant application proposes the construction and production of Epo-Ig fusion proteins (all claims), including Epo/IgG-Fc and Epo/IgG-CH fusion proteins (Claims 90 and 139), with biological activities (EC₅₀'s) that are comparable to wild-type EPO on a molar basis, which is encompassed by all of the EC₅₀ values claimed (Claims 90 and 139). The grant sections describe joining the C-terminus of EPO to the N-terminus of human IgG-Fc or IgG-CH domains using a restriction site to join the protein domains (Claims 90, 139 and dependents therefrom).

More specifically, the grant sections provided show the amino acid sequence and structural organization of human EPO and provide details for the production of the constructs using unique restriction sites to join the EPO and IgG-Fc or IgG-CH sequences. On page 11, lines 14-22 of the application, we state that the junction sequences (peptide linkers) will be the only non-natural sequences in the fusion protein, thereby teaching the use of a linker to connect the proteins (Claims 90, 92-94, and 132-135). We describe how we will amplify the genes encoding EPO and the Ig portions of the fusion protein using, *e.g.*, PCR, and clone them into mammalian cell expression vectors (Claim 104). We discuss how to express proteins in mammalian COS or CHO cells (Claim 104). We state that we expect the proteins to be secreted as homodimers, and we state that we will purify the proteins by affinity chromatography using protein A columns (Claims 102, 104, 105). We state that bioactivities of the proteins can be measured using the UT7/epo cell line and again, propose EPO-Ig fusions with biological activities comparable to wild-type EPO (Claims 90 and 139).

Evidence of Diligence beginning prior to July 24, 1998

(1) As evidence of diligence beginning prior to July 24, 1998, enclosed as Exhibit B are documents showing that after the filing of the grant application, and prior to July 24, 1998, we monitored the progress of the grant application. On or prior to July 24, 1998, we received communications from the granting agency including a grant priority score, a summary statement containing reviewer critiques, and a letter indicating that the agency intended to fund the grant pending resolution of the time commitment and employment issues.

(2) As evidence of diligence beginning prior to July 24, 1998, enclosed as Exhibit C are relevant portions of notebook pages that show that we were diligently performing experiments to reduce the invention as claimed in Claims 90-94, 96, 102, 104, 105, 130-135 and 139 to practice. Specifically, these notebook pages, spanning dates from June 3, 1998 to July 15, 1998 describe the planning and execution of experiments to amplify the genes encoding EPO.

Namely BB41 and BB42 oligonucleotides for PCR cloning Epo cDNA were designed and obtained, and Quickclone cDNA was obtained. PCR cloning was attempted and the first attempt was unsuccessful (notebook pages 97, 98 and 103-106).

An attempt to make RNA from human Hep 3B cells (which had been reported to express Epo under hypoxic conditions) is described on notebook pages 107-112.

An attempt to use RT-PCR to amplify Epo cDNA from human liver RNA (unsuccessfully) is shown on notebook pages 15-16.

PCR primers BB45 and BB46 for cloning Epo were ordered (page 113). A new PCR primer BB47 was also ordered. (page 113). PCR amplified of Epo cDNA from Hep 3B RNA using BB45 and BB47 oligos was successful. (pages 34-35). The Epo cDNA was cloned into pUC19 plasmid DNAs and sent to Macromolecular Resources in Fort Collins for DNA sequencing (page 34-42). Epo clone #10 had the correct DNA sequence and was called plasmid pBBT131, pUC19::Epo E10 (page 42).

(3) As further evidence of diligence prior to July 24, 1998, enclosed as Exhibit D are documents showing that we also contacted researchers regarding the UT7/Epo cell line and how to obtain it. We received an email from the researcher who isolated the cell line confirming that a second researcher had permission to send us the UT7/Epo cell line.

Evidence of Continued Diligence and Constructive and/or Actual Reduction to Practice

(1) After July 24, 1998 we were taking steps to reduce the invention as claimed in Claims 90-94, 96, 102, 104, 105, 130-135 and 139 to practice. We ordered human TF1 cell line from ATCC for testing Epo proliferation. We also ordered recombinant human Epo from R&D Systems, Inc. for testing TF-1 cell line response. Monkey COS cells were ordered from ATCC for transient transfection experiments. See Exhibit E.

(2) After July 24, 1998, we responded to the granting agency regarding the employment and time commitment, and the grant funding began, also shown in Exhibit F.

(3) Exhibit G represents an experiment completed October 1-9, 1998 that provides evidence that we were diligently performing experiments to reduce the invention as claimed in Claims 90-94, 96, 102, 104, 105, 130-135 and 139 to practice. Specifically, these notebook pages describe the planning of different EPO-Ig fusion constructs and then the initial production of constructs encoding immunoglobulin and EPO proteins that were used to produce various EPO-Ig fusion proteins. These constructs were used to produce the linkered fusion proteins. Specifically, these notebook pages show diagrams for fusion constructs of EPO and IgG₁ and IgG₄ (Fc or hinge and CH domains), as well as marked up sequences for EPO and IgG₁ and IgG₄, and oligonucleotide design, as well as experiments showing successful cloning and expression of the individual components that were used to create EPO-Ig fusion proteins as claimed.

(4) Exhibit H also represents an experiment completed on January 12-15, 1999 which provides evidence that we were diligently performing experiments to reduce the invention as claimed in Claims 90-94, 96, 102, 104, 105, 130-135 and 139 to practice. In particular, this experiment establishes that we were diligently performing experiments to reduce to practice the construction, production, and testing of biologically active EPO-Ig fusions, beginning with EPO-Ig fusions having the recited small peptide linkers consisting of serine and glycine amino acids.

The three fusion proteins are denoted pBBT 179, pBBT 180 and pBBT 181. Construct pBBT 179 is EPO-IgG₁/CH₁ (EPO-IgG₁-CH); construct pBBT 180 is EPO-IgG₁/hinge (IgG₁-Fc); and construct pBBT 181 is EPO-IgG₄/hinge (IgG₄-Fc). In these fusions, the peptide linker was 7 amino acids in length (Claims 90, 92, 133, 134, 135, 136, 138 and 139) and consisted of glycine and serine residues (Claims 90, 92, 133, 134, 135, 136, 138 and 139). The Ig portion of the fusion protein was either IgG1-Fc, IgG4-Fc or IgG1-CH, demonstrating that we had produced fusion proteins using two different Ig isotypes and both Fc and CH fusions. Prior to the experiment described in Exhibit H below, the three fusion protein nucleotide constructs were produced using recombinant techniques, and the fusion proteins were expressed by transfecting a host cell with an expression vector comprising the recombinant constructs, culturing the host cell under conditions effective to express the fusion protein, and harvesting the fusion protein expressed by the host cell (Claim 104). The expression of the fusion proteins was demonstrated and levels of expression quantitated by Western blots using anti-EPO antibodies in order to determine the concentrations of the proteins prior to putting them into the bioassay described in Exhibit H below (data not shown in this Declaration).

The EC₅₀'s (as defined in the present application, the concentration of protein required for half-maximal stimulation) of each of the three EPO-Ig fusion proteins was measured in the UT7/epo cell proliferation bioassay, shown in the notebook pages of Exhibit H. The first page of the Exhibit shows the three fusion proteins tested as discussed above (BBT 179, BBT 180, BBT 181), with plasmid pCDNA3.1 serving as a negative control and a wild type human EPO protein purchased from R & D Systems, Inc. serving as a positive control. Plasmids BBT179, BBT180 BBT181 and pcDNA3.1 were used to transfect COS cells and the conditioned media containing the fusion proteins was harvested several days later. Serial 10-fold dilutions of the conditioned media were prepared and assayed in the UT7/epo cell proliferation assay. The serial dilutions were called tubes 1-6. Estimated concentrations of the EPO-IgG fusion proteins in the assay were: tube 1, 0.0005 ng/mL; tube 2, 0.005 ng/mL; tube 3, 0.05 ng/mL; tube 4, 0.5 ng/mL; tube 5, 5 ng/mL; and tube 6, 50 ng/mL. Six serial dilutions of the EPO control protein were prepared as well. Concentrations of the EPO standard in the assay were: tube 1, 0.0004 ng/mL; tube 2, 0.004 ng/mL; tube 3, 0.04 ng/mL; tube 4, 0.4 ng/mL; tube 5, 4 ng/mL and tube 6, 40 ng/mL. The second page of Exhibit H shows the experimental set-up for each of the three test plates (called plates A, B and C). Each of the serial dilutions was assayed in triplicate. Additional control wells contained no cells (called "no cells") or just media but no Epo or Epo-Ig (called "0"). The UT7/epo cell line shows a strong proliferative response to rEPO, as evidenced by a dose-dependent increase in absorbance values, which is proportional to cell number. In the absence of rEPO, the majority of UT7/epo cells die, giving absorbance values less than 0.1. The results shown on the third-sixth pages of the Exhibit provide the raw data and graphs of the activity of the three EPO-Ig fusion proteins as compared to rEPO and a negative control. The graphs plot absorbance of the wells on the Y-axis versus the dilution tube on the X-axis (the percent of the COS cell supernatant (% sup) in the dilution tubes for the fusion proteins or the number of EPO units/mL (1 unit = 8 ng/mL) in the dilution tubes also is plotted on the X-axis under the appropriate dilution tube). The dilution tube closest to the EC₅₀ for each fusion protein was tube 4, which contained an estimated 0.5 ng/mL of the fusion protein. The serial dilution tube closest to the EC₅₀ for the EPO control protein also was tube 4, which contained an estimated 0.4 ng/mL of EPO. Thus, the EC₅₀s of the fusion proteins were within the scope of less than 4 ng/ml, and comparable to (within at least 4 fold) activity of wild type EPO on a molar basis, which also

represents an EC₅₀ of less than 1000 ng/ml and less than 10 ng/ml (Claims 96, 134, 135, and 136).

(5) Between January 15, 1999 and July 13, 1999, we continued to design constructs and perform experiments to produce and test additional EPO-Ig fusion proteins as claimed in Claims 67, 68, 77, 78, 80-87, 89-94, 96, 102, 104, 105, and 125-138, and we worked with patent counsel to constructively reduce the invention to practice by the preparation and filing of U.S. Provisional Application No. 60/143,458, filed July 13, 1999, which is the priority document for the present application. The following Exhibits describe activities and representative experiments that pertained to the constructive and actual reduction to practice of the invention during this time period.

Exhibit I contains notebook pages dated from February 9-17, 1999, showing the results of an experiment that began on January 21, 1999 (see reference to 1/21/99 transfection), showing the larger scale transfection of host cells with the EPO-Ig fusion constructs described in the Exhibits above and the purification of the fusion proteins (purification of EPO-IgG₄/hinge (Fc) or pBBT 181, EPO-IgG₁/CH or pBBT 179, and EPO-IgG₁/hinge (Fc) or pBBT 180 is shown).

(6) Exhibit J contains a notebook page date February 26, 1999, showing the design and beginning of the construction of a recombinant construct encoding a different EPO-Ig fusion protein, which was an EPO joined at its carboxy-terminus to IgG₄-CH (an immunoglobulin domain that does not contain a variable region) using a linker comprised of serine and glycine residues. Note the diagram illustrating the construct plan.

(7) Exhibit K contains notebook pages showing an experiment beginning on March 23, 1999, and ending on April 28, 1999, describing the COS cell transfection, expression and purification of the new EPO-Ig fusion protein (EPO-IgG₄/CH (pBBT 185)) described in Exhibit J above.

(8) Exhibit L provides relevant portions of a grant application completed and filed on April 14, 1999, which includes particular experimental details regarding the construction and production of EPO-Ig fusion proteins having 2 and 4 amino acid linkers, and EPO-Ig fusions having no intervening linker. This grant also includes much of the material described in the grant sections of Exhibit A and presents the data shown in Exhibit H and related experiments and specifically, describes the detailed construction, production and testing of EPO-IgG-Fc and EPO-

Ig-CH fusions with a peptide linker. This document also describes specific methods for separating monomers from dimers.

(9) Exhibit M contains notebook pages showing the results on June 8-9, 1999, of an experiment that began April 30, 1999, showing the purification of a large scale EPO-IgG₁-Fc fusion protein (pBBT 180) transfection experiment.

(10) Exhibit N contains a notebook page from an experiment performed on July 6-9, 1999, showing the bioactivity for the EPO-IgG₄-CH fusion protein (pBBT 185) and for the scaled up expression of the EPO-IgG₁-Fc fusion protein (pBBT 180). The EC₅₀s for the fusion proteins are shown as compared to wild-type recombinant EPO. The EC₅₀ for the EPO control protein was 0.48 and 0.48 ng/mL in two assays; the EC₅₀s for BBT185 were 2 and 2.1 ng/mL in two assays and the EC₅₀s for BBT180 were 1.3 and 1.3 ng/mL in two assays.

(11) Exhibit O is U.S. Provisional Application Serial No. 60/143,458, filed July 13, 1999, which constructively reduces to practice the invention as claimed in Claims 90-94, 96, 102, 104, 105, and 130-135.

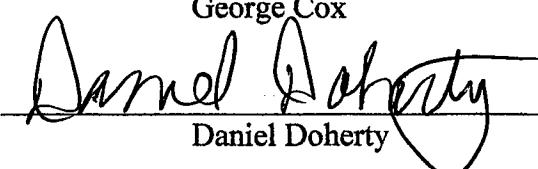
4. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date

4/08/09

Date

George Cox


Daniel Doherty